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Molecular assay (Polymerase Chain Reaction based technology) for identification of cheating and adulteration of marketable Canned beef, Handmade sausage, Kofta and Hawawshi samples in Qalubia Governorate, Egypt

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ABSTRACT

Adulteration of meat products attracted the interest of food safety groups, and it plays a significant part in environmental forensics. Meat products are a good source of protein since they include all essential amino acids and nutrients for humans. Adulteration in meat products is defined as the fraudulent substitution or addition of lower-cost animal or plant proteins, as well as the mislabeling of actual constituents. So, species identification of animal food origin is an important aspect of its control in several countries that are growing concerned about the number of serious food crimes committed by organized criminals. A total of 48 different commercial meat product samples (Canned beef, Handmade sausages, Kofta and Hawawshi samples) Twelve of each were randomly collected from Qalubia Governorate supermarkets and analyzed by Polymerase Chain Reaction (PCR) technique. We found Canned beef were free from adulteration while Handmade sausages, Kofta and Hawawshi samples were adulterated by chicken meat (31.2%), canine (4.2%), and equine (8.3%) meat. The present results concluded that PCR assay is the unique test for detecting adulteration of some meat products. Moreover, canned beef strictly undergoes inspection and food control while Handmade meat products used low–cast and undeclared meat because they don't undergo strict food control.

1. INTRODUCTION

Adulteration of meat products can take a variety of forms, such as intentionally substituting ingredients, replacing higher quality meat with lower quality meat, such as low-fat muscle tissue with higher fat content tissues, offal meat or skin, and, last but not least, mechanically recovered meat, or completely replacing meat with a non-meat substance, usually in the form of plant-based flour. Adulteration of beef products is a global issue for legal, economic, religious, and public health or medical reasons. Food forensics comprises identifying meat species, but it also entails preserving meat quality and assisting in the enforcement of regulations in many countries, which is a more difficult and innovative task. Furthermore, for fair-trade support, adequate labeling is essential. Branding, product promotion, and laws may all contribute to the inclusion of more informative label information (Ballin, 2010). The flesh used in meat products experiences considerable morphological changes because of the grinding process, and as a result of this circumstance, some manufacturers are more prone to engage in fraudulent operations from an economic aspect. Adulteration can occur in canned beef, sausage, and meat balls that have been rendered out by adding flavor and spices, smoking, fermenting, salting, and curing, or other methods to enhance flavor or preserve the product (WHO, 2015). Since meat species adulteration is a worldwide problem that violates food labeling regulations, the species name of meats used to make meat products must be disclosed on the product label. Furthermore, food legislation prohibits the sale of other meat species with fictitious names to increase profit, and more descriptive label information is required (Ballin, 2010; Hassanien et al., 2018). Canned beef products are frequently available, they are regular meals that are excellent for working, they're also quite easy to make. Spices, soy protein, starch, nitrite, salt, ascorbates, and phosphate can all be added to chopped beef or comminuted form as a fundamental raw material (Abdullah, 2007). Handmade sausage is a popular lunch among people of all ages, especially children and teenagers. In the meantime, is a typical Egyptian meat dish comprised mostly of minced beef flesh, fat tissues, dried rusk, salt, and spices (Shahin,2016). Kofta meatballs are often created by emulsifying fine ground beef with a starch, then seasoning with salt and herbs (Purnomo,1990). Hawawshi is a popular Egyptian street snack consisting of ground beef seasoned with delicious warm spices, onions, and garlic. In Egypt, street vended food, particularly (Handmade sausage, Kofta, and Hawawshi) ready-to-eat street vended meat products sandwiches, may pose a health risk because to the poor

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conditions in which they are prepared, such as utilizing low-quality meat and lack of quality control legislation. Food safety labs are having trouble discriminating between raw material species utilized in industrial food manufacturing and recognizing animal species in food outputs (Luo et al., 2008). So far, several protein-based analytical approaches have been used to detect meat product fraud, but they are time-consuming, costly, and inadequately specific, whereas DNA-based techniques are more reliable, faster, and less expensive (Yin et al., 2009). The ubiquity, amount, and stability of DNA in all cells have all been demonstrated to be good targets for identifying meat species, which is a benefit of DNA-based analysis (Pascoal et al., 2005; Edris et al., 2012). Matsunaga et al. (1999) mentioned Mitochondrial DNA (mtDNA) molecules combined with polymerase chain reaction (PCR) provide a fast, sensitive, and highly specific alternative to protein-based methods, and the PCR extraction process is less time-consuming and technically challenging than the previous method. Even in complex meals, PCR has proven to be a viable method for determining minute levels of different species (Mafra et al., 2008). Furthermore, Ghovvati et al. (2009) and Girish and Nagappa (2009) stated that with standard multiplex PCR, many targets can be amplified at the same time, allowing for the identification of many species in a short period of time. The presence of target DNA was successfully recognized in all the species studied, and the amplification was unaffected by the addition of spices or the cooking process. Egypt (El-Sangary and Gabrail 2006; Abd El-Nasser et al., 2010) is one of the countries where meat products have been replaced. Aim of present study detection of adulteration of some meat products in Qalubia Governorate, Egypt by using PCR technique.

2. MATERIAL AND METHODS

1. Samples collection

A total of 48 distinct commercial beef meat products (canned beef, Handmade sausage, kofta and Hawawshi) were collected at random from markets in Egypt's Qalubia Governorate. If not processed right away, all samples were delivered to the laboratory in a refrigerated box and immediately frozen at -20 ℃ for the next procedures.

2. Samples preparation:

2.1. DNA extraction

According to Obrovska et al. (2002) The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract mitochondrial DNA from samples, with some modifications to the manufacturer's recommendations.25 mg of the grind sample was treated overnight at 56°C with 20 µl of proteinase K and 180 µl of ATL buffer. After incubation, 200 µl of ATL buffer was added to the lysate, which was then incubated for 10 minutes at 72°C before receiving 200 µl of 100 % ethanol. The lysate was then centrifuged after being transferred to a silica column. Following the manufacturer's instructions, the sample was washed and centrifuged. The nucleic acid was eluted with 100 µl of the kit's elution buffer.

2.2. Primer design

Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (1).

2.3. PCR amplification

According to Jain et al.(2007) primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

2.4. Analysis of the PCR Products.

PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. The fragment sizes were determined using the Generuler 100 base pair (bp) ladder (Fermentas, Germany) (tables 1&2). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra). Photos (1-4), The data was then evaluated using computer software.

Table 1 Primers sequences, target genes, amplicon sizes

Species	Target gene	Primers sequences	Amplified segment (bp)	Reference
Chicken	125 rRNA	TGAGAACTACGAGCACAAAC GGGCTATTGAGCTCACTGTT	445	Dalmasso et al. 2004
Canine	cytB	GGAGTATGCTTGATTCTACAG AGAAGTGGAATGAATGCC	808	Abdel-Rahman et al., 2009
Equine	mtDNA	CCCTCAAACATTTCATCATGATGAAA GCTCCTCAAAAGGATATTTGGCCTCA	359	Maede., 2006
Pork	12S Rrna-tRNA Val	CTACATAAGAATATCACCCAC ACATTGTGGGATCTTCTAGGT	290	Tasara et al., 2005

Table 2 Primer cycling conditions during PCR

Species	Amplification (35 cycles)				
	Primary Denaturation	Secondary denaturation	Annealing	Extension	Final extension
Chicken	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.
Canine	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.
Equine	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.
Pork	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.

3. RESULTS

PCR was planned to amplify partial genes differing in amplicon sizes for the identification of different meat species table (1). The obtained results indicated successful amplification of the target (125 rRNA, cytB, mtDNA and 12S Rrna-tRNA Val) gene sequences, the Amplification of chicken, canine, equine, and pork's meat genomic DNA with species-specific oligonucleotide primers revealed amplicon sizes, table (1) (445, 808, 290, and 359) bp respectively, with Photos (1-4) respectively. The results showed multiplex PCR methods recorded that 12 Canned beef samples were negative for all types of adulteration, as shown in Photograph (1-4) and table (3). Otherwise, there were 6 samples out of 12 Handmade sausage samples (50%) were adulterated by chicken meat but negative for other adulterants as shown in Photograph (1) and table (2). While 6 samples out of 12 Kofta samples (50%) were adulterated by chicken meat and 4 samples out of the same12 kofta samples (33.3%) were adulterated by equine meats as shown in Photograph (1) and (3) respectively and table (2). In the other side 3 samples out of 12 Hawawshi samples (25%)

were adulterated by chicken meat and one sample out of the same 12 Hawawshi samples (8.33%) was adulterated by canine meat, as shown in Photograph (1 and 2) respectively and Table (2), but they were free from adulteration with pork and equine meat.

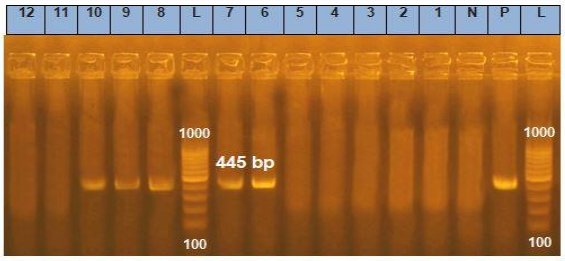

Photo 1 Agarose gel electrophoresis of PCR amplicon (445 bp) of mitochondrial 12S rRNA gene amplification for chicken adulteration showing positive in samples at lane 6,7,8,9 and 10. Lane M, 1kb plus DNA ladder.

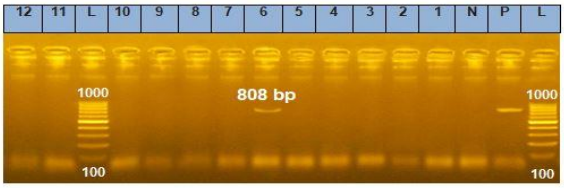

Photo 2 Agarose gel electrophoresis of PCR amplicon (808 bp) of cytB gene amplification for canine meat adulteration showing positive in samples at lane 6. Lane M, 1kb plus DNA ladder

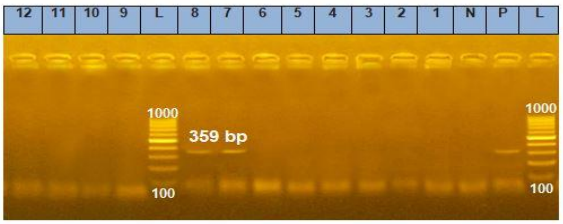

Photo 3 Agarose gel electrophoresis of PCR amplicon (343 bp) of mtDNA gene amplification for equine meat adulteration showing positive in samples at 7 and 8. Lane M, 1kb plus DNA ladder.

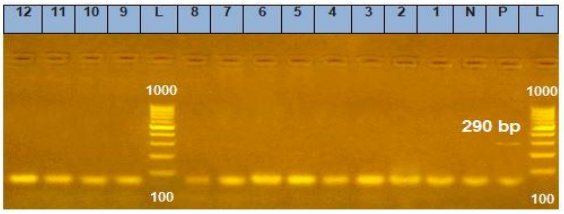

Photo 4 Agarose gel electrophoresis of PCR amplicon (290 bp) of mitochondrial 12S Rrna-rRNA Val gene amplification for pork meat adulteration showing negative in all examined samples, 1kb plus DNA ladder

Table 3 Incidence of adulteration of Canned Beef, Handmade Sausage, Kofta and Hawawshi samples by using PCR sample number (48 samples 12 of each product).

	Canned beef		Handmade Sausage		Kofta		Hawawshi		Total	
	12		12		12		12		48	
	No	%	No	%	No	%	No	%	No	%
Chicken meat	0	0	6	50	6	50.00	3	25.00	15	31.25
Canine meat	0	0	0	0	0	0	1	8.33	1	2.10
Equine meat	0	0	0	0	4	33.3	0	0	4	8.33
Pork meat	0	0	0	0	0	0	0	0	0	0.00
Total	0	0	6	50	10	83.3	4	33.33	20	41.66

4. DISCUSSION

Adulteration of meat products, defined as the mixing of declared meat from other animals with beef meat, has been documented in several countries, including Egypt. Meat adulteration includes the use of canine, horse, and pork intermixed with beef meat. In the Islamic religion, canine, horse and pork meat are prohibited for human consumption (Haram) (Unajak et al., 2011), also, their presence implies poor hygienic conditions for manufacture and assimilation as a human health concern, as well as combining beef with chicken, which is a global problem. Moreover, beef DNA was not found in beef sausage samples in Turkey, while chicken and turkey DNA was found in items branded as 100 % beef (Ali and Hamid 2014). The main reason for this type of adulteration is not only the lower cost of chicken meat compared to beef, but also the use of chicken waste products, such as fat connective tissue, blood vessels, nerves, cartilage, sinew, bloody effluvia, and even bone fragments mixed with meat and used as adulterants. In comparison to meat, these waste products have a lesser nutritional value and may be contaminated with foodborne pathogens. As a result, the presence of these microorganisms in final products due to insufficient cooking temperature poses a possible health risk to consumers (Ayaz and Erol 2006; Doosti et al., 2011). So, species identification is becoming more prevalent and necessary, Immunodiffusion, immunoelectrophoresis, isoelectric focusing, and DNA amplification were utilized to identify meat species (Meyer et al., 1996; Koh et al., 1998). Polymerase Chain Reaction (PCR) technology has proven to be more reliable and sensitive than previous techniques in looking for species adulteration and substitution of meat products with chicken or other forbidden meat. DNA amplification has proven to be more reliable and sensitive than previous techniques in looking for species adulteration and substitution of meat products with chicken or other forbidden meat (Zahran and Hagag 2015). Adulteration of Handmade sausage, kofta, and Hawawshi with unidentifiable meat sources, low-quality, and banned meat has been linked to some Egyptian native plants (Hassanin et al, 2018). So, we looked for chicken, canine, equine and pork meat in these meat products in our present study by using PCR technique. The present results indicated that Canned beef were free from adulteration may be due to canned beef strictly undergo inspection and food quality control. While, our results displayed that Handmade sausages samples adulterated by chicken meat (50%),kofta samples adulterated by chicken meat(50%) and by equine meat (33.3%) ,while Hawawshi samples were adulterated by chicken meat (25%) and by canine meat (8.33%) but negative for pork meat ,these results may be due to small native handmade plants in local areas used low–cast and undeclared meat either lower price of chicken meat instead of beef or chicken waste products, trimmings. These findings matched those of (Abd El-Nasser et al., 2010) found that 7 % of minced meat has been adulterated with donkey meat, and sausage has been adulterated with donkey meat (8 %). In Gharbia governorate, equine flesh was identified in all samples of kabab, grilled kofta, and meat loaves, and 4% of the analyzed meat loaves were contaminated with canine meat, according to El-Shewy (2007) and Hamouda et al. (2020) identified dog meat in 4 (33.3%) of raw kofta samples and 8 (66.75%) of Balady sausage samples, and (Abbas et al., 2014) discovered that 6 (8.82%) of 68 fermented sausages contained Haram (illegal or banned) meat. PCR analysis of 96 beef meat and meat product samples collected at random from street vendors and prominent retail markets (24 burgers, 16 minced meat, 24 kofta, 16 sausage, 7 raw meat, and 9 luncheon) revealed 6 positives for donkey meat (3 from sausage, 2 from minced meat, and 1 from kofta) and two positives for horse flesh (from sausage) (Khaled et al., 2019) . PCR technology was employed by Hassanin et al.

(2018) discovered that 4 samples of minced meat (26.67 %) and 4 samples of sausage (26.67 %) were adulterated by cattle and horse meat, and 3 samples (20 %) were tainted by pure equine meat. In the case of minced meat and sausage samples acquired from processing factories, 15 samples (100%) and 13 samples (86.67%) were found to be contaminated, respectively, and 0% of minced meat was found to be adulterated. Furthermore, (Mehdizadeh et al., 2014) used species-specific (PCR) to detect adulteration of chicken meat in uncooked hamburgers, finding that undeclared chicken meat was found in 94.4 % of all hamburgers, including 100% of homemade and 89.6 % of factory samples. Multiplex PCR was used to evaluate different meat samples by Ghovvati et al. (2009), and the results revealed that chicken flesh was identified in 40% of the sausages and 30% of the cold cut samples, which in the same line with (Ahmed et al., 2011), higher adulteration rates were found in beef burgers with chicken at 69 %, raw kofta with pork at 45.5 %, and donkey at 18 % in Upper Egyptian local markets using the PCR method. PCR analysis of 40 total commercial beef product samples (Omran et al., 2019) revealed 87.5 % adulteration and mislabeling with one or more species. They were predominantly blended with chicken meat or by-products (72.5%), next donkey (12.5%), and finally human tissue (2.5%), which was discovered in manually produced kofta samples. Another study in Egypt's Suez Canal cities used species-specific PCR (Mosaad, 2017) discovered sheep, chicken, and equine species in 80 %, 50 %, and 10% of the studied oriental sausage samples, respectively, as well as the absence of beef meat in 20% of the samples. The present results revealed that 20 samples of 48 samples (41.66%) were adulterated by undeclared meat rather than in labels, while according to statistics given by Ballin et al. (2009) on meat product mislabeling, 20% of meat products in the US, 22% in Turkey, 15% in Switzerland, and 8% in the UK were mislabeled. Also, 95 (68 %) of 139 samples of processed meat products from South African retail outlets and butcher shops contained species not stated on the label (Cawthorn et al., 2013). Many meat products contained traditional species such as donkey, goat, and buffalo. From our findings indicating that food quality control legislation by PCR diminish meat products adulteration, as Canned beef hadn't adulterated, while Handmade products without food quality control were expected to be adulterated. In the near past we used to detected meat products that were adulterated with equine and pork meat , but now we detected meat products that are adulterated with canine meat. This could be due to our frustration with street vendors because we have inadequate control over the Handmade food.

5. CONCLUSION

PCR is unique methods for species determination used to monitor meat adulteration in any meat products, it can be employed in a more advanced laboratories, such as a Forensic and Quality control laboratories, Governments must employ this technology as a routine inspection on a strict and regular basis to assess meat fraud in meat products in Egypt specially local areas under veterinary authority in Egyptian accredited agency ,because species-specific PCR does not necessitate the use of expensive devices such as real-time PCR analyses, it is a cost-effective method., and also deterrent penalties should be enacted for anyone who has meat adulteration crimes. Also consumers wariness must be increased about of meat adulteration, so consumers must purchase their requirement of meat and meat products from well-known sources and restaurants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper

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